phosphatase activity found in homogenates prepared with the Waring blender. (The activity in the fractions isolated by centrifugation was higher but still lower than in Waring blender preparations.) Convinced that this represented an error in the assay, he stored the fractions in a refrigerator. When he repeated the assay five days later it was unexpectedly an order of magnitude greater in all fractions and in the range expected from Waring blender preparations in the mitochondrial fraction.[42] He concluded that the activity of the enzyme must have been masked in the fresh preparation and only activated over time.

Soon de Duve pursued the latency in the enzyme activation and proposed that upon initial fractionation the acid phosphatase was contained within a separate "baglike" particle that limited its access to the substrate. The rough treatment in the Waring blender or the gradual aging of the homogenate prepared for cell fractionation released the enzyme from this container. Only once released was it possible to assay its activity. This readily explained why acid phosphatase did not destroy the various phosphate compounds found elsewhere in the living cell. The fact that, with the four-fraction technique, aged mitochondrial fractions yielded the highest levels of acid phosphatase activity suggested that the mitochondrion itself was the sac housing the digestive enzymes (Berthet & de Duve, 1951). De Duve discovered that it was not the mitochondrion when the high-speed attachment to de Duve's centrifuge broke and François Appelmans, a medical student working with him, had to prepare mitochondria using an ordinary preparatory centrifuge with longer centrifugation times. The resulting mitochondria showed no acid phosphatase activity. This led de Duve to continue fractionation, segregating a light fraction containing acid phosphatase and a heavy fraction containing cytochrome oxidase (Berthet et al., 1951; de Duve & Berthet, 1954).

At the Second International Congress of Biochemistry in Paris in 1952, de Duve presented his claim that acid phosphatase belonged to a special cytoplasmic particle. Afterward, P. G. Walker, a British biochemist, related to him that he had found similar results with β-glucuronidase (Walker, 1952); de Duve then tested his light fraction and discovered that it contained β-glucuronidase as well. Subsequently he investigated several other enzymes – acid ribonuclease, acid deoxyribonuclease, cathepsin, urate

[42] In a historical rendition of the events, de Duve (1969, p. 7) wrote, "we could have rested satisfied with this result, dismissing the first series of assays as being due to one of those troublesome gremlins that so often infest laboratories, especially late at night . . . Two factors saved us. . . . The assays had been repeated with the old as well as with fresh reagents, giving identical results. The gremlin, if he was the culprit, must have been a very subtle one. Furthermore, we had noted that the greatest discrepancy between the two series of results occurred in the mitochondrial fractions, the smallest one in the supernatant fraction."